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APPLICANT: Steinman et al.

EXAMINER: Schwadron, Ronald B.

SERIAL NO.: 09/925,284

ART UNIT: 1644

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FOR: ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE
IMMUNE RESPONSE THEREFROM

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
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ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, MICHEL NUSSENZWEIG, hereby declare and state that:

1. I am a Howard Hughes Investigator, Sherman Fairchild Professor and Senior Physician at Rockefeller University having received my Ph.D. degree from the Rockefeller University in 1981 and my M.D. degree from New York University in 1982. I received postdoctoral medical and scientific training at Harvard University. My full curriculum vitae is attached hereto as Exhibit A.

2. My principal area of research is in Immunology and among other positions I serve as reviewer in numerous funding agencies of many countries, including the National Institute of Health, March of Dimes, Dana Foundation. I also have served as reviewer for numerous scientific journals, and I am the Editor of the Journal of Experimental Medicine and the Journal of Immunologic Methods.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM", having U.S. Serial Number 09/925,284, which is a continuation-in-part of U.S. application Serial Number 09/586,704, filed on June 5, 2000, which is a continuation of U.S. Serial Number 08/381,528, filed on January 31, 1995, now abandoned.

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4. I have reviewed the disclosure of the present application, with particular emphasis on the support in the application as filed for the preparation and generation of antibodies against human and mouse DEC-205 proteins.

5. The present application claims a method of enhancing the development of tolerance to a pre-selected antigen for which tolerance is desired in a mammal comprising exposing ex vivo or in vivo dendritic cells from said mammal to a conjugate comprising said pre-selected antigen covalently bound to an anti-human DEC-205 antibody or an anti-murine DEC-205 antibody that binds to human DEC-205 under conditions that promote dendritic cell quiescence. More particularly, the pre-selected antigen is selected from the group consisting of allergens, autoantigens and antigens participating in allograft rejection. The human DEC-205 protein has a carboxy terminal and amino terminal amino acid sequence as disclosed in the parent application, U.S. Serial Number 09/586,704, filed on June 5, 2000, as SEQ ID NOs: 1 and 2, respectively. Furthermore, as noted in the parent application USSN 09/586,704, the first 19 amino acid residues of the amino terminal human DEC-205 protein (designated as SEQ ID NO: 13 in the parent application) were used to generate antibodies that reacted with human DEC-205. The sequences from the parent application have now been included in the sequence listing for the present application and are designated as SEQ ID NO: 7 for the carboxy terminal, SEQ ID NO: 8 for the amino terminal, and SEQ ID NO: 9 for the first 19 amino acids of the amino terminal used for antibody generation.

6. The subject matter of the present application was based on work performed in my laboratory, whereby the human DEC-205 molecule was cloned and expressed (Guo, M., Gong, S., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., Nussenzweig, M.C. & Steinman, R.; (2000), A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells, *Human Immunology* 61:729-738). Anti-human DEC-205 antibodies were then prepared by immunizing animals with the first 19 amino acid residues from the N terminal fragment of the cloned human DEC-205 protein.

7. To summarize briefly, the cloning of human DEC-205 was done through use of a cDNA fragment of the 3' portion of mouse DEC-205. This was used to screen a human lymphocyte and thymus cDNA library using standard procedures known to those skilled in the art. In particular, the cDNA fragment of mouse DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5'-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were identified by DNA sequencing on

both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dyc determinator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371). The human cDNAs were expressed in pEF-BOS modified to carry a 3' human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and FcII domains were amplified from plasmids by PCR using 5' MG31 primers and 3' MG35 primers. The 5' -- primer contains a SpeI site, while the 3' -- primer contains a NotI site and codes for PRR at the junction point of DEC-205 and the Fc tag. The human DEC-205 Fc fusion protein was produced by transiently transfecting 293 cells using calcium phosphate mediated gene transfer. The fusion protein was purified on protein A sepharose and was then used to inject mice. Following several booster injections, the serum was tested for antibodies that reacted with the CR-FcII domain of the human DEC-205 molecule using Western blot procedures. Afterwards, the spleens were harvested from those animals showing a positive reaction and were fused with SP2/0 cells. The supernatants were screened by ELISA, dot blot, thymus tissue staining and FACS analysis. Cell clones that secreted anti-human DEC-205 antibodies were further subcloned and expanded.

8. The present application teaches methods for inducing tolerance by conjugating an antigen to a DEC-205 antibody for targeting to the DEC-205 receptor on specific cells, such as dendritic cells, under conditions that promote dendritic cell quiescence. The antibodies that react with the DEC-205 proteins, in particular, the anti-human DEC-205 antibodies, were prepared using the first 19 amino acid residues from the amino terminal end of the cloned human DEC-205 protein, as described in the parent application U.S. Serial Number 09/586,704, and further attested to in this declaration. Thus, it is my belief that the disclosure of the present application provides sufficient written description for a person skilled in the art to prepare such antibodies that react with human DEC-205 protein as presently claimed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: 1/3/05

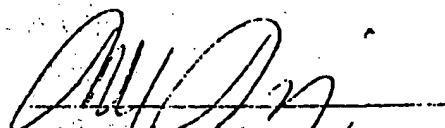

Michel Nussenzweig, M.D., Ph.D.



EXHIBIT A

CURRICULUM VITAE

Name: Michel C. Nussenzweig

Date of Birth: February 10, 1955

Education:

1975 B.A. - New York University College of Arts and Sciences
1981 Ph.D. - The Rockefeller University
1982 M.D. - New York University School of Medicine

Clinical Training:

1982-1985 Intern & Resident, Internal Medicine
Massachusetts General Hospital
1984-1985 Clinical Fellow, Infectious Diseases
Massachusetts General Hospital

Postdoctoral Training:

1986-1989 Harvard Medical School, Department of Genetics

Professional Appointments

1990-1996 Assistant & Associate Professor, The Rockefeller University
1990-1999 Assistant & Associate Investigator, Howard Hughes Medical Institute
1996-present Professor & Senior Physician, The Rockefeller University
1999-present Investigator, Howard Hughes Medical Institute
2000-present Sherman Fairchild Professor of Immunology, The Rockefeller Univ.

Honors & Awards

Summa Cum Laude, New York University College of Arts and Sciences - 1975; Phi Beta Kappa, New York University College of Arts and Sciences - 1975; Alpha Omega Alpha, New York University Medical School - 1982; Bertram M. Gresner Memorial Research Award, New York University School of Medicine - 1982; Elected Member American Society of Clinical Investigators - 1997; Solomon A. Berson Award for Basic Science - 2002

Teaching:

Immunology, Course Organizer

Institutional:

Chair, Transgenic Facility Coordinating Committee
Chair, Animal Care and Use Committee

Chair, Hospital Seminar Committee
Member, Immunology Search Committee
Member, Institutional Review Board for Biohazards, Radioisotopes, Toxic Chemicals, and Carcinogens
Member Hospital GCRC Scientific Advisory Committee
Elected Senior Faculty Representative Academic Council
Member, Virology Search Committee

National

Arthritis Foundation Molecular Immunology study section 1993-1996
NIH Immunobiology Study Section Ad Hoc reviewer 1998, and 1999
NIH ALY Study Section Ad Hoc Reviewer, 1999
NIH NIAID Council Ad Hoc 1998
Organizer Keystone Symposium on Dendritic Cells 1998
Organizer Keystone Symposium on B Cells 1999
March of Dimes Review Committee 1999-
External Reviewer LMGD NICHD 2000
Damon Runyon Cancer Research Fund Review Committee 2000-2002
American Association of Immunologists Program Committee 2000-
NIH ALY Study Section Member 2001-
Organizer Keystone Symposium on B Cell Biology 2003

Editorial:

1996-Present	Editor, The Journal of Experimental Medicine
1999-Present	Editor, The Journal of Immunological Methods
2000-Present	Transmitting Editor, International Immunology
2002-Present	Advisory Editor, Nature Reviews Immunology

Consultant:

Abgenix, Fremont, CA
Zycos, Lexington MA

Professional Memberships:

American Association of Immunologists
American Medical Association
The New York Academy of Sciences
Kunkel Society
Harvey Society

Publications:

1. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Chen, L.L. & Cohn, Z.A. Dendritic Cells: an important new cell type in the mixed leukocyte reaction. In: Kaplan JG, ed. Proc. of the 13th International Leukocyte Culture conference. *Elsevier/North Holland Publ. Co.* 273. (1979)
2. Steinman, R.M., & Nussenzweig, M.C. Dendritic cells features and functions. *Immunol. Rev.* 53:127-147. (1980)
3. Nussenzweig, M.C. & Steinman, R.M. Contribution of dendritic cells to stimulation of the syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196-1212. (1980)
4. Nussenzweig, M.C., Steinman, R., Gutchinov, B., & Cohn, Z.A. Dendritic cells are accessory cell for the development of anti-trinitrophenyl cytotoxic T cells. *J. Exp. Med.* 152:1070-1084. (1980)
5. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Chen, L.L., Schlesinger, S.J., & Cohn, Z.A. Dendritic Cells of the Mouse Identification and Characterization. *J. Invest. Dermatol.* 75:14-16. (1980)
6. Nussenzweig, M.C., Steinman, R.M., Unkeless, J.C., Witmer, M., Gutchinov, B., & Cohn, Z.A. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J. Exp. Med.* 154:168-187. (1981)
7. Steinman, R.M., Witmer, M.D., Nussenzweig, M.C., Gutchinov, B., & Austyn, J.M. Studies with a monoclonal antibody to mouse dendritic cell. *Transplantation* 31:151. (1981)
8. Nussenzweig, M.C. & Steinman, R.M. Surface of dendritic cells. *Immunology Today* 3:65 (1982)
9. Nussenzweig, M.C., Steinman, R.M., Witmer, M.D., & Gutchinov, B. A monoclonal antibody specific for mouse dendritic cells. *Pro. Natl. Acad. Sci., USA* 79:161-165. (1982)
10. Steinman, R.M., Gutchinov, B., Witmer, M.D., & Nussenzweig, M.C. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613-627. (1983)
11. Nussenzweig, M.C., Shaw, A.C., Sinn, E., Danner, D.B., Holmes, K.L., Morse, H.C., & Leder, P. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin. *Science* 236:816-819. (1987)

12. **Nussenzweig, M.C., Shaw, A.C., Sinn, E., Torres, J., & Leder, P.** Allelic exclusion in transgenic mice that carry human mutant human immunoglobulin genes. *J. Exp. Med.* 167:1969-1974 (1988)
13. **Nussenzweig, M.C., Schmidt, E.V., Shaw, A.C., Sinn, E., Torres, J., Mathey-Prevot, B., Pattengale, P.K. & Leder, P.** A human immunoglobulin gene reduces the incidence of lymphomas in c-Myc bearing transgenic mice. *Nature* 336:446-450. (1989)
14. **Morton, C.C., Nussenzweig, M.C., Sousa, R., Sorenson, G.D., Pettengill, O.S. & Shows, T.B.** Mapping and characterization of an X-linked processed gene related to mycll. *Genomics* 4:367-375. (1989)
15. **Shimizu, A., Nussenzweig, M.C., Tataunobu-Ryushin, M., Leder, P., & Honjo, T.** Immunoglobulin double isotype expression by trans-mRNA in a human immunoglobulin transgenic mouse. *Proc. Natl. Acad. Sci. USA* 86:8020-8023. (1989)
16. **Shimizu, A., Kinashi, T., Nussenzweig, M.C., Tataunobu-Ryushin, M., Leder, P., & Honjo, T.** Molecular mechanism for immuno-globulin double-isotope expression. *Cold Spring Harbor Symposia on Quantitative Biology* Vol. LIV 175.
17. **Friedlander, R., Nussenzweig, M.C., & Leder, P.** Complete nucleotide sequence of the membrane form of the human IgM heavy chain. *Nucleic Acids Res.* Vol. 18(14):4278. (1990)
18. **Shimizu, A., Nussenzweig, M.C., Han, H., Sanchez, M., & Honjo, T.** Trans-splicing as a Possible Molecular Mechanisms for the Multiple Isotope Expression of the Immunoglobulin Gene. *J. Exp. Med.* 173:1385-1393. (1991)
19. **Costa, T., Suh, H-Y., & Nussenzweig, M.C.** Chromosomal Position of Rearranging Gene Segments Influences Allelic Exclusion in Transgenic Mice. *Proc. Natl. Acad. Sci. USA* 89:2205-2208. (1992)
20. **Costa, T., Franke, R.R., Sanchez, M., Misulovin, Z., & Nussenzweig, M.C.** Functional Reconstitution of an Immunoglobulin Antigen Receptor in T Cells. *J. Exp. Med.* 175:1669-1676. (1992)
21. **Sanchez, M., Misulovin, Z., Burkhardt, A.L., Mahajan, S., Costa, T., Franke, R.R., Bolen, J.B. & Nussenzweig, M.C.** Signal Transduction by Immunoglobulin Is Mediated Through Ig α and Ig β . *J. Exp. Med.* 178:1049-1055 (1993)
22. **Burkhardt, A.L., Costa, T., Misulovin, Z., Stealy, B., Bolen, J.B., & Nussenzweig, M.C.** Ig α and Ig β are Functionally Homologous to the Signaling Protein of the T cell Receptor. *Molecular & Cellular Biology* Vol. 14(2):1095-1103. (1994)

23. Kurosaki, T., Muta, T., Sanchez, M., Misulovin, Z., **Nussenzweig, M.C.**, & Ravetch, J.V. A 13 amino acid motif in the cytoplasmic domain of FcγRIIB modulates B cell receptor signalling. *Nature* Vol. 368:70-73. (1994)
24. Spanopoulou, E., Roman, C.A.J., Corcoran, L., Schlissel, M.S., Silver, D.P., Storb, U., Nemazee, D., **Nussenzweig, M.C.**, Shinton, S.A., Hardy, R.R., & Baltimore, D. Expression of functional immunoglobulin transgenes allows ordered B-cell differentiation to progress in Rag-1 deficient mice. *Genes & Development* Vol. 8:1030-1042. (1994)
25. Wayne, J., Suh, H., Misulovin, Z., Sokol, K.A., Inaba, K., & **Nussenzweig, M.C.** A Regulatory Role for Recombinase Activating Genes, RAG-1 and RAG-2, in T Cell Development. *Immunity* Vol. 1:95-107. (1994)
26. Qin, X.F., Luo, Y., Suh, H.-Y., Wayne, J., Misulovin, Z., Gerster, T., Roeder, R.G. & **Nussenzweig, M.C.** Transformation by homeobox genes can be mediated by selective transcriptional repression. *EMBO J.* Vol. 13:5967-5976. (1994)
27. Wayne, J., Suh, H.-Y., Sokol, K., Petrie, H.T., Witmer-Pack, M., Edelhoff, S., Disteche, C.M., & **Nussenzweig, M.C.** TRC Selection and Allelic Exclusion in RAG Transgenic Mice that Exhibit Abnormal T Cell Localization in Lymph Nodes and Lymphatics. *J. Immunol.* Vol. 153:5491-5502. (1994)
28. Mitchell, R.N., Barnes, K.A., Grupp, S.A., Sanchez, M., Misulovin, Z., **Nussenzweig, M.C.** & Abbas, A.K. Intracellular Targeting of Antigens by Membrane Immunoglobulin in B Lymphocytes *J. Exp. Med.* 181:1705-1714. (1995)
29. Heufler, C., Humborg, C., Steinman, R.M., Schuler, G. and **Nussenzweig, M.C.** A molecular strategy to identify molecules that are specific for dendritic cells and/or critical to their unique immunostimulatory function. *Adv. Exp. Med Biol.* 378:367-369 (1995)
30. Papavasiliou, F., Misulovin, Z., Suh, H.-Y., **Nussenzweig, M.C.** The role of Igβ in Precursor B cell Transition and Allelic Exclusion. *Science* Vol. 268:408-411 (1995)
31. Jiang, W., Swiggard, W., Heufler, C., Peng, M., Steinman, R.M., & **Nussenzweig, M.C.** The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 375:151-155. (1995)
32. Inaba, K., Swiggard, W.J., Inaba, M., Meltzer, J., Mirza, Sasagawa, T., **Nussenzweig, M.C.**, & Steinman, R.M. Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse Leukocytes. *Cell. Immunol.* 163:148-156 (1995)
33. Swiggard, W.J., Asra, M., **Nussenzweig, M.C.**, & Steinman, R.M. DEC-205, a 205 kDa Protein abundant on mouse dendritic cells and thymis epithelium that is detected by the

- monoclonal antibody NLDC-145: Purification, characterization and N-terminal amino acid sequence. *Cell. Immunol.* 165:302-311 (1995)
34. Papavasiliou, F., Jankovic, M., Suh, H.-Y., **Nussenzweig, M.C.** The Cytoplasmic Domains of Ig α and Ig β can Independently Induce the Precursor B cell Transition and Allelic Exclusion. *J. Exp. Med.* 182(5):1389. (1995)
 35. Gong, S., & **Nussenzweig, M.C.** Regulation of an Early Developmental Checkpoint in the B cell Pathway by Ig β . *Science* 272:411-414 (1996)
 36. Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., **Nussenzweig, M.C.**, & Li, G.C. Requirement for Ku80 in Growth and V(D)J Recombination. *Nature* 382:551-555 (1996)
 37. Kim, U., Qin, X., Gong, S., Stevens, S., Luo, Y., **Nussenzweig, M.C.**, & Roeder, R.G. The B cell specific transcription coactivator OCA-B is essential for normal production of immunoglobulin isotypes. *Nature* 383:542-547 (1996)
 38. Papavasiliou, F., Jankovic, M., & **Nussenzweig, M.C.** Surrogate or Conventional Light Chains Are Required for Membrane Immunoglobulin Mu to Activate the Precursor B Cell Transition. *J. Exp. Med.* 184:2025-2030 (1996)
 39. Gong, S., Sanchez, M. & **Nussenzweig, M.C.** Counterselection against Du Is Mediated through Immunoglobulin (Ig) α -Ig β . *J. Exp. Med.* 184:2079-2084 (1996)
 40. Cortes, P., Weis-Garcia, F., Misulovin, Z., Nussenzweig, A., Lai, J.S., Li, G., **Nussenzweig, M.C.**, & Baltimore, D. In vitro V(D)J recombination: signal joint formation. *Proc. Natl. Acad. Sci. USA* 93: 14003-13 (1996)
 41. Sawchuk, D.J. Weis-Garcia, F. Malik, S., Besmer, E. Bustin, M. **Nussenzweig, M.C.** & Cortes, P. V(D)J recombination: Modulation of RAG1 and RAG2 cleavage activity on 12/23 substrates by whole cell extract and DNA bending proteins. *J. Exp. Med.* 185:2025-2032 (1997)
 42. Weis-Garcia, F., Besmer, E. Yu, W., Sawchuk, D., Hu, Y., **Nussenzweig, M.C.** & Cortes, P. V(D)J recombination: In vitro coding joint formation. *MCB* 17:6379-6385(1997)
 43. Papavasiliou, F., Jankovic, M., Gong, S. & **Nussenzweig, M.C.** Control of immunoglobulin gene rearrangements in developing B cells, review. *Current Opinions in Immun.* 9:233-244 (1997)
 44. **Nussenzweig, M.C.** Tails to teach a B cell. *Current Biology.* (1997)

45. Nussenzweig, M.C. & Allison, Jim. Lymphocyte activation and effector functions. How signals are integrated in the immune system (Editorial Overview) *Current Opinions in Immun.* 9:293-295 (1997)
46. Papavasiliou, F., Casellas, R., Suh, H., Qin, X., Besmer, E., Pelanda, R. Nemazee, D., Rajewsky, K., and Nussenzweig, M.C. V(D)J Recombination in Mature B cells: a Mechanism for Altering Antibody Responses. *Science* 278:298-301 (1997)
47. Honghai, O., Nussenzweig, A., Kurimasa, A., Soares, V., Li, X., Cordon-Cardo, C., Li, W., Cheong, N., Nussenzweig, M.C., Iliakis, G., Chen, D.J. & Li, G.C. Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination in vivo. *J. Exp. Med.* 186:921-929 (1997)
48. Lee, S.Y., Reichlin, A., Santana, A., Sokol, K.A., Nussenzweig, M.C. & Choi, Y. TRAF2 is essential for JNK but not NF-KB activation and regulates lymphocyte proliferation and survival. *Immunity* 7:703-713 (1997)
49. Casellas, R., Nussenzweig, A., Wuerffel, R., Pelanda, R., Reichlin, A., Suh, H., Qin, X-F., Besmer, E., Kenter, A., Rajewsky, K. and Nussenzweig, M.C. Ku80 is required for immunoglobulin isotype switching. *EMBO Vol 17* 8:2404-2411 (1998)
50. Meffre, E., Papavasiliou, F., Cohen, P., de Bouteiller, O., Bell, D., Karasuyama, H., Schiff, C., Banchereau, J., Liu, Y-J., and Nussenzweig, M.C. Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B cells. *J. Exp. Med.* 188:765-772 (1998)
51. Qin, X-F., Reichlin, A., Luo, Y., Roeder, R.G. and Nussenzweig, M.C. OCA-B integrates B cell antigen receptor-, CD40L-, and-IL-4-mediated signals for the germinal center pathway of B cell development. *EMBO* 17:5066-5075 (1998)
52. Besmer, E., Mansilla-Soto, J., Cassard, S., Sawchuk, D., Brown, G., Sadofsky, M., Lewis, S., Nussenzweig, M.C. and Cortes, P. Hairpin coding end opening by the recombination activating genes RAG1 and RAG2. *Molecular Cell* 2:817-828 (1998)
53. Qin, X-F., Schwers, S., Yu, W., Papavasiliou, F., Suh, H., Nussenzweig, A., Rajewsky, K., and Nussenzweig, M.C. Secondary V(D)J recombination in B-1 cells. *Nature* 397:355-359 (1999)
54. Nussenzweig, M.C. Immune Receptor Editing: Revise and Select. *Cell* 95:875-878 (1998)
55. Yu, W., Nagaoka, H., Jankovic, M., Misulovin, Z., Suh, H., Rolink, A., Melchers, F., Meffre, E., & Nussenzweig, M.C. Continued RAG expression in late stages of B cell development and no apparent re-induction after immunization. *Nature* 400:682-687 (1999)

56. Yu, W., Misulovin, Z., Suh, H., Hardy, R.R., Jankovic, M., Yannoutsos, N & Nussenzweig, M.C. Coordinate regulation of RAG1 and RAG2 by cell type-specific DNA elements 5' of RAG2. *Science* 285:1080-1084 (1999)
57. Tiefenthaler, M., Marksteiner, R., Neyer, S., Koch, F., Hofer, S., Schuler, G., Nussenzweig, M.C., Schneider, R., & Heufler, C. M1204, a Novel 2',5' Oligoadenylate Synthetase with a Ubiquitin-Like Extension, Is Induced During Maturation of Murine Dendritic Cells^{1,2}. *Journal of Immun.* 163:760-765 (1999)
58. Santagata, S., Besmer, E., Villa, A., Bozzi, F., Allingham, J.S., Sobacchi, C., Haniford, D.B., Vezzoni, P., Nussenzweig, M.C., Pan, Z-Q., & Cortes, P. The RAG1/RAG2 complex a 3' flap endonuclease: implications for junctional diversity in V(D)J and transpositional recombination. *Molecular Cell* 4:935-947 (1999)
59. Yu, W., Nagaoka, H., Misulovin, Z., Meffre, E., Suh, H., Jankovic, M., Yannoutsos, N., Casellas, R., Besmer, E., Papavasiliou, F., Qin, X., & Nussenzweig, M.C. RAG Expression in B Cells in Secondary Lymphoid Tissues. Cold Spring Harbor Symposia on Quantitative Biology Vol. LXIV:207-210, (1999)
60. Nagaoka, H., Yu, W. & Nussenzweig, M.C. Regulation of RAG expression in developing lymphocytes. *Current Opinion in Immunology* 12:187-190 (2000)
61. Kronin, V., Wu, L., Gong, S., Nussenzweig, M.C., & Shortman, K. DEC-205 as a marker of dendritic cells with regulatory effects on CD8 T cell responses. *International Immunology* 12(5):731-735 (2000)
62. Liu, Y., Chirino, A.J., Misulovin, Z., Leteux, C., Feizi, T., Nussenzweig, M.C., & Bjorkman, P.J. Crystal structure of the cysteine-rich domain of mannose receptor complexed with a sulfated carbohydrate ligand. *J. Exp. Med.* 191(7):1105-1115 (2000)
63. Leteux, C., Chai, W., Loveless, R.W., Yuen, C-T., Uhlin-Hansen, L., Combarnous, Y., Jankovic, M., Maric, S.C., Misulovin, Z., Nussenzweig, M.C. & Feizi, T. The cysteine-rich domain of the macrophage mannose receptor is a multispecific lectin that recognizes chondroitin sulfates A and B and sulfated oligosaccharides of blood group Lewis^a and Lewis^x types in addition to the sulfated N-glycans of lutropin. *J. Exp. Med.* 191(7):1117-1126 (2000)
64. Difilippantonio, M.J., Zhu, J., Chen, H.T., Meffre, E., Nussenzweig, M.C., Max, E.E., Ried, T. & Nussenzweig, A. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404:510-514 (2000)
65. Nagaoka, H., Gonzalez-Aseguinolaza, G., Tsuji, M. & Nussenzweig, M.C.

Immunization and infection change the number of recombination activating gene (RAG)-expressing B cells in the periphery by altering immature lymphocyte production. *J. Exp. Med.* 191(12):2113-2120 (2000)

66. Mahnke, K., Guo, M., Lee, S., Sepulveda, H., Swain, S.L., Nussenzweig, M.C. & Steinman, R.M. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via MHC II+, lysosomal compartments. *J. of Cell Biology* 151:673-683 (2000)
67. Meffre, E., Davis, E., Schiff, C., Cunningham-Rundles, C., Ivashkiv, L.B. Staudt, L.M., Young, J.W. & Nussenzweig, M.C. Circulating human B cells that express surrogate light chains and edited receptors. *Nature Immunology* 1(3):207-213 (2000)
68. Guo, M., Gong, S., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., Nussenzweig, M.C. & Steinman, R. A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Human Immunology* 61:729-738 (2000)
69. Reichlin, A., Hu, Y., Meffre, E., Nagaoka, H., Gong, S., Kraus, M., Rajewsky, K., & Nussenzweig, M.C. B cell development is arrested at the immature B cell stage in mice carrying a mutation in the cytoplasmic domain of Ig β . *J. Exp. Med.* 193(1):13-23 (2001)
70. Kraus, M., Pao, L.I., Reichlin, A., Hu, Y., Canono, B., Cambier, J.C. Nussenzweig, M.C. & Rajewsky, K. Interference with Ig α ITAM phosphorylation modulates or blocks B cell development, depending on the availability of an Ig β cytoplasmic tail. *J. Exp. Med.* 194(4):455-470 (2001)
71. Meffre, E., Casellas, R., Nussenzweig, M.C. Antibody regulation of B cell development. *Nature Immunology* 1(5):379-385 (2000)
72. Casellas, R., Yang Shih, T-A., Kleinewietfeld, M., Rakonjac, J., Nemazee, D., Rajewsky, K. & Nussenzweig, M.C. Contribution of receptor editing to the antibody repertoire. *Science* 291:1541-1544 (2001)
73. Wurbel, M-A., Malissen, M., Guy-Grand, D., Meffre, E., Nussenzweig, M.C., Richelme, M., Carrier, A., and Malissen, B. Mice lacking the CCR9 -chemokine receptor show a mildly impeded of early T and B cells and a reduction in TCR⁺ gut intraepithelial lymphocytes. *Blood* 98(9):2626-2632 (2001)
74. Meffre, E., Chiorazzi, M. and Nussenzweig, M.C. Circulating human B cells that express surrogate light chains display a unique antibody repertoire. *J. Immunol.* 167(4):2151-2156 (2001)
75. Meffre, E. Catalan, N., Seltz, F., Fischer, A., Nussenzweig, M.C.* and Durandy, A. Somatic hypermutation shapes the antibody repertoire of memory B cells in humans. *J. Exp. Med.* 194(3):375-378 (2001)

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76. Yannoutsos, N., Wilson, P., Yu, W., Chen, H.T., Nussenzweig, A., Petri H. and **Nussenzweig, M.C.** The role of recombination activating gene (RAG) reinduction in thymocyte development in vivo. *J. Exp. Med.* 194(4):471-480 (2001)
77. Meffre, E., Milili, M., Blanco-Betancourt, C., Antunes, H., **Nussenzweig, M.C.*** and Schiff, C. Immunoglobulin heavy chain expression shapes the B cell receptor repertoire in human B cell development. *J. Clin. Invest.* 108(6):879-886 (2001)

*Equal contributing senior author

78. Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M. and **Nussenzweig, M.C.** Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med.* 194(6):769-779 (2001)
79. Petersen, S., Casellas, R., Reina San-Martin, B., Chen, H.T., Difilippantonio, M.J., Wilson, P.C., Hanitsch, L., Celeste, A., Muramatsu, M., Pilch, D.R., Redon, C., Ried, T., Bonner, W.M., Honjo, T., **Nussenzweig, M.C.**, and Nussenzweig, A. AID is required to initiate Nbs1/γ-H2AX focus formation and mutations at sites of class switching. *Nature* 414:660-665. (2001)
80. Steinman, R.M. and **Nussenzweig, M.C.** Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci.* 99(1):351-358 (2002)
81. Pelanda, R., Braun, U., Hobeika, E., **Nussenzweig, M.C.** and Reth, M. Ig-α and Ig-β are not required for the development of B cell progenitor cells and the rearrangement of IgH chain genes. *J. of Immunology* 169(2):865-872 (2002)
82. Lee, S.J., Evers, S., Roeder, D., Parlow, A.F., Risteli, J., Risteli, L., Lee, Y.C., Feizi, T., Langen, H. and **Nussenzweig, MC.** Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science* 295(5561):1898-901 (2002)
83. Shih, T.A., Roederer, M. and **Nussenzweig, M.C.** Role of antigen receptor affinity in T cell-independent antibody responses in vivo. *Nat Immunol* 3(4):399-406 (2002)
84. Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola V., Meffre, E., Difilippantonio, M.J., Redon, C., Pilch, D.R., Oлару, A., Eckhaus, M., Camerini-Otero, R.D., Tessarollo, L., Livak, F., Manova, K., Bonner, W.M., **Nussenzweig, M.C.**, Nussenzweig, A. Genomic Instability in Mice Lacking Histone H2AX. *Science* 296:922-927 (2002)

85. Shih, T. A., Meffre, E., Roederer, M. and Nussenzweig M.C. Role of BCR affinity in T cell dependent antibody responses *in vivo*. *Nat. Immunol.* 3(6):570-575 (2002)
86. Guy-Grand, D., Azogui, O., Celli, S., Darche, S., Nussenzweig, M.C., Kourilsky, P., and Vassalli, P. Extrathymic lymphopoiesis: ontogeny and contribution to gut intraepithelial lymphocytes in athymic and euthymic mice. *J. Exp. Med.* 197(3):333-341 (2003)
87. Lee, S.J, Gonzalez-Aseguinolaza, G. and Nussenzweig, M.C. Disseminated Candidiasis and Hepatic Malarial Infection in Mannose-Binding Lectin-A Deficient Mice. *Mol. & Cellular Biology* Vol. 22:8199-8203 (2002)
88. Casellas, R., Jankovic, M., Meyer, G., Gazumyan, A., Luo, Y., Roeder, R.G., and Nussenzweig, M.C. OcaB is Required for Normal Transcription and V(D)J Recombination of a Subset of Immunoglobulin κ Genes. *Cell* Vol. 110:575-585 (2002)
89. Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M. C., and Steinman, R.M. Efficient Targeting of Protein Antigen to the Dendritic Cell Receptor DEC-205 in The Steady State Leads to Antigen Presentation on Major Histocompatibility Complex Class I Products and Peripheral CD8(+) T Cell Tolerance. *J Exp Med.* 196(12):1627-1638. (2002)
90. Meffre E, Nussenzweig, M.C. Deletion of immunoglobulin beta in developing B cells leads to cell death. *Proc Natl Acad Sci USA* 99(17):11334-11339 (2002)
91. Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21:685-711. (2003)
92. Lee S.J., Zheng, N-Y, Clavijo M, Nussenzweig M.C. Normal host defense during systemic candidiasis in mannose receptor-deficient mice. *Infect Immun.* 71(1):437-45. (2003)
93. Ramiro A.R., Stavropoulos P., Jankovic M, Nussenzweig M.C. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. *Nat Immunol.* 4(5):452-456 (2003)
94. Reina-San-Martin, B., Difilippantonio, S., Hanitsch, L., Masilamani, R., Nussenzweig, A. and Nussenzweig, M.C. γ -H2AX is required for recombination between immunoglobulin switch regions but not for intra-switch region recombination or somatic hypermutation. *J Exp Med.* 197(12):1767-1778 (2003)
95. Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., and Nussenzweig, M.C. Predominant autoantibody production by early human B cell precursor. *Science* 301:1374-1377 (2003)

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